VASCULAR BUDDING IN BOTRYLLOIDES [1, 2]

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In our previous paper (1957) it has been demonstrated that in *Botryllus primigenus*, in addition to the ordinary palleal (peribranchial) budding, new buds are formed also from aggregations of blood-cells at the base of ampullae, and the epithet "vascular" has been proposed for that kind of budding. The question at once arises whether this vascular budding occurs also in other members of the family Botryllidae. Our researches along this line have revealed that *Botrylloides violaceum* under certain circumstances propagates by vascular budding entirely analogous to that of *Botryllus*.

In this brief note the process of vascular budding in *Botrylloides* will be described and then compared with that of *Botryllus*.

Materials and Methods

The materials on which the following observations were made were living colonies of *Botrylloides violaceum* Oka, commonly found in the vicinity of Shimoda Marine Biological Station, Shimoda, Japan. As is well known, in *Botrylloides* the zooids are arranged in meandering systems instead of in circular systems as in *Botryllus*.

To facilitate observation, colonies were fixed on glass slides. The technique used for fixing the colonies, setting out the slides in the bay, etc. was essentially the same as that described in the paper of Oka and Usui (1944). Observations on living materials were supplemented, if necessary, with examination of sections.

We take this opportunity and express our thanks to the Director and staff of the Station for providing us facilities for carrying out this research. Thanks are also due to Miss Yoshiko Oshima for her helpful assistance in laboratory works.

Observations

Developmental cycle in the colony of Botrylloides

Developmental cycle in the colony of *Botrylloides* is exactly the same as in *Botryllus*. In both, the zooids in a colony are perfectly coordinated, so we can speak of the phases of a colony as a whole. A colony has four successive phases, which constitute a developmental cycle. For particulars see our previous paper (1957).

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[2] The cost of this research has been partly covered by the Scientific Research Expenditure of the Department of Education of Japan.

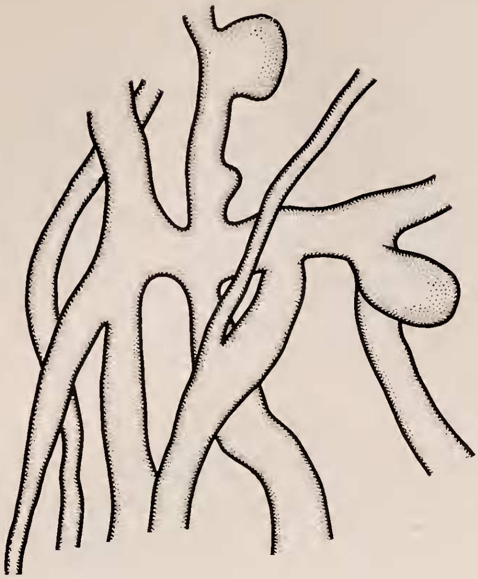

FIGURE 1. Test vessels immediately after isolation. × ca. 60

Conditions for the appearance of vascular buds

In the normal growth of the colony of *Botrylloides violaceum,* buds are formed exclusively from the palleal wall, *i.e.,* no sign of vascular budding is seen. When, however, a small piece containing ampullae and vessels but no zooids is cut out from the marginal part of a colony, vascular buds appear in it after 2 or 3 days.

In *Botrylloides* colonies, as in *Botryllus* colonies, numerous blood vessels traverse the test (Fig. 1) and terminate in contractile ampullae at the periphery of the

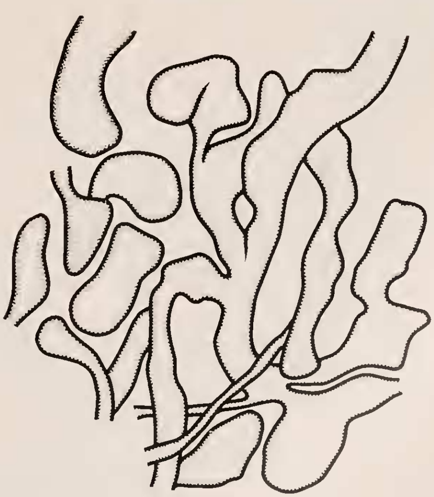

FIGURE 2. Test vessels in a one-day-old piece. × ca. 60

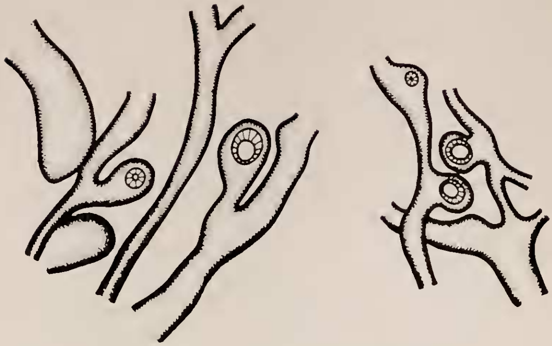

FIGURE 3. Vascular buds in a 3-day-old piece. × ca. 60

FIGURE 4. Vascular buds in a 4-day-old piece. × ca. 60

FIGURE 5. Section of a bud from a 2-day-old piece. × ca. 2000

colony. In a cut-out piece, the whole vascular system, inclusive of ampullae, strongly contracts. At the same time many club-shaped branches are sent out, and finally a dense network of anastomosing vessels is formed (Fig. 2). It is seen that a flow of blood is maintained in it. On such vessels the vascular buds are formed.

Formation of the vascular bud and its further development

It is seen in sections that the formation of the vascular bud is initiated by gathering of lymphocytes (diameter ca. 3–4 μ) under the epidermis of the blood vessel (Fig. 5). The initial number of lymphocytes is about 15–20 as in *Botryllus*. The development of a new zooid out of this cell mass follows exactly the same course as in *Botryllus*. At first, through intensive cell division a hollow blastula-like structure (diameter ca. 30–40 μ) is formed (Fig. 6); at the same time, the local epidermis of

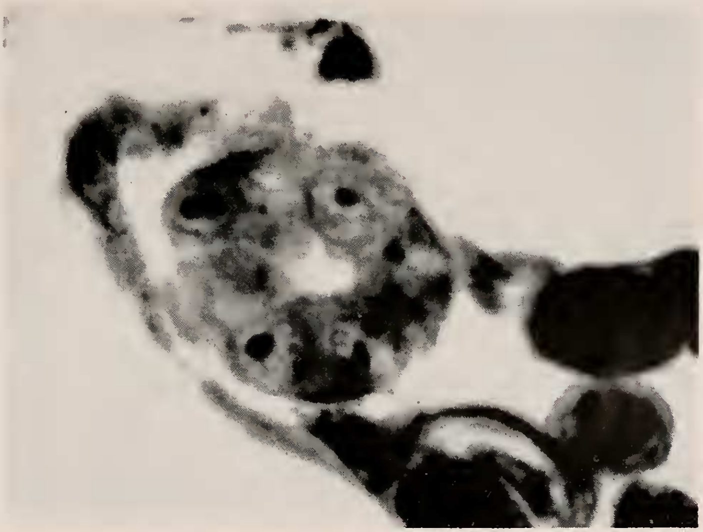

FIGURE 6. Section of a bud from a 3-day-old piece. $\times$ ca. 2000

the blood vessel gradually protrudes so as to wrap up the vesicle in itself (Fig. 3). The bud is now distinctly visible as such even in living materials. Morphologically, the vascular bud of this stage (diameter 50 μ) corresponds to the palleal bud of stage 3 except that it has no ova. Further development is the same as in the palleal bud. As an example, buds in a 4-day-old piece are shown in Figure 4.

Time of appearance

At whatever phase in the developmental cycle of the colony isolation may occur, vascular budding always begins two to three days after isolation and lasts about 24 hours. This means that vascular budding is not related to any definite phase of the original colony.

Vascular budding is strictly restricted to that period, for, as it seems, further bud-formation from the vascular wall is inhibited by growing vascular buds, and the new zooids, once formed by vascular budding, propagate exclusively by palleal budding.

Site of appearance

At the time of budding, the vascular system in the isolated piece is represented by a network of blood vessels, the diameter of which varies from 45 μ to 120 μ. Unlike *Botryllus*, the vascular buds never appear at the bases of ampullae. Nor do they appear on the newly formed club-shaped endings. They are always formed along the walls of the old blood vessels.

Degeneration of the buds

At the end of the budding period, *i.e.*, 3 to 4 days after isolation, we could often count 60-70 newly formed buds, but not all of them continued to develop. As in the case of *Botryllus*, only those which surpass a certain size can develop and form perfect zooids, while the remaining ones undergo involution. An example of various sizes of buds is shown in Table I.

TABLE I

Various sizes of vascular buds in a piece 4 days after isolation

Diameter (in μ)	Number of buds
ca. 10	9
ca. 15	8
ca. 20	25
ca. 30	17
ca. 40	6
ca. 50	1
ca. 60	1
ca. 70	1
ca. 80	1
ca. 90	1
Total	70

Of these 70 buds, those larger than 40 μ continued to develop (11 buds, or 16%), while those under 30 μ soon began involution and finally disappeared without leaving any traces behind (59 buds, or 84%). In another case, of 35 buds once formed, only 6 (17%) developed into perfect zooids and formed a colony with two systems. Thereupon the colony began to grow by palleal budding.

DISCUSSION

Differences between Botryllus and Botrylloides

The formation of the bud itself is precisely alike in both forms. Yet, as to the conditions for and the time and site of the appearance of the vascular buds, there are differences between *Botryllus primigenus* and *Botrylloides violaceum* as stated below:

1. In *Botryllus*, vascular budding occurs in active colonies concomitantly with palleal budding. In *Botrylloides*, vascular budding is never seen under normal

conditions. Only when a small piece of a colony devoid of zooids is isolated, vascular budding is, so to speak, evoked in it.

2. In *Botryllus* the appearance of the vascular buds is limited to a certain phase (late phase B) in the developmental cycle of the colony. In *Botrylloides* vascular buds can be formed at any phase of the original colony.

3. In *Botryllus* the buds are located strictly at the bases of the ampullae, while in *Botrylloides* the buds are scattered across the colony along the walls of the vascular system.

Actually all these differences are the same as existing between *Botryllus primigenus* and *Botrylloides gascoi* except on one point. In *Botrylloides violaceum* we could repeatedly observe vascular budding in isolated pieces. In *Botrylloides gascoi,* however, an isolated piece devoid of zooids never regenerated a colony, and none of the ampullae in such a piece showed the least tendency towards budding (Bancroft, 1903, p. 451). Probably this led Bancroft to suppose that the presumable vascular buds observed by him in an aestivating colony were developed not from vessels but from the zooids of the original colony before these had degenerated entirely and later severed their connections with the parent zooids. It is to be hoped that some future investigator will repeat the experiments with *Botrylloides gascoi.*

Regulation acting upon the vascular buds

Botryllids are known for their zooids being most perfectly coordinated.

In *Botryllus,* the vascular buds are formed a little later than the corresponding palleal buds, but they are soon synchronized with these. Buds formed too late are forced to degenerate, thus being eliminated from the colony. All this regulating influence is supposed to come from the pre-existing active zooids.

In *Botrylloides,* the vascular buds are formed in the absence of any pre-existing zooids. Yet, sooner or later, all the newly formed zooids are synchronized, and, as in *Botryllus,* buds formed too late are eliminated from the colony. Possibly with the growth of early buds a new coordinating system is established in the piece and this regulates the growth of late-coming buds.

Budding in Botryllidae

Now that vascular budding has been demonstrated also in *Botrylloides,* it is to be assumed that this kind of budding is rather widely distributed among the family Botryllidae.

It is generally believed that stolonial budding—of which vascular budding is only a special form—is a rather primitive type of budding, while palleal budding is phylogenetically a relatively late acquisition. Moreover, palleal budding is so unique in nature that it cannot be derived from any other known kind of budding. So it has been assumed "that the primitive pleurogonid, undoubtedly derived from an enterogonid, had already lost any such capacity for budding, and that within the new order the Botryllinae have re-acquired it by a new method" (Berrill, 1950, pp. 50–51). That the original capacity for budding has not been completely lost in botryllids is clear from our investigations on *Botryllus* and *Botrylloides.* Only, with the rise of the new method of palleal budding, it has been more and more suppressed. In *Botryllus primigenus* vascular budding still takes part, though concomitantly with palleal budding, in the natural growth of the colony. In

Botrylloides violaceum, and probably *Botrylloides gascoi,* vascular budding is totally suppressed in the ordinary life of the colony. Only in the absence of zooids the otherwise latent capacity of forming buds from the walls of the blood vessels is called forth as a means to save the colony from extinction.

Summary

1. Generally *Botrylloides violaceum* Oka propagates by palleal budding alone. Only when a small piece of a colony devoid of zooids is isolated, new buds are formed from the walls of the test vessels, *i.e.*, vascular budding appears.

2. As in *Botryllus,* these new buds are formed from aggregations of lymphocytes under the wall of the test vessels.

3. Unlike *Botryllus,* the buds are not bound to any definite sites, but are distributed irregularly along the walls of the vascular system.

4. The buds generally appear 2–3 days after isolation, at whatever phase of the original colony the isolation may occur.

5. Major difference between *Botryllus* and *Botrylloides* is that in the former vascular budding coexists with palleal budding, while in the latter vascular budding is totally suppressed in the normal life of the colony.

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